

Title: NOVEL LITAF BINDING SITE PEPTIDES AND  
METHODS OF USING THE SAME  
Applicant(s): Amar et al  
Client/Matter No.: 50047/019002  
Filing Date: March 10, 2004      Serial No.: Not Yet Assigned  
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**(a) hLITAF/pGEX4T-1 constructs**

- Diagram illustrating the GST fusion constructs for hLITAF fragments:

  - 1. hLITAF aa 1-228: GST + Peptide A
  - 2. hLITAF aa 1-75: GST + Peptide A (aa 1-75)
  - 3. hLITAF aa 1-151: GST + Peptide A (aa 1-151)
  - 4. hLITAF aa 76-151: GST + Peptide B (aa 76-151)
  - 5. hLITAF aa 76-228: GST + Peptide B (aa 76-228)
  - 6. hLITAF aa 152-228: GST + Peptide C (aa 152-228)
  - 7. hLITAF aa 152-228 Δ164-180: GST + Peptide C (aa 152-180)
  - 8. hLITAF aa 152-228 Δ181-195: GST + Peptide C (aa 152-195)

**(b) hTNF- $\alpha$  Promoter/pGL3-basic constructs**

1. wt TNF $\beta$ -pGL3basic .991  
(-991 to 1)  
NF- $\kappa$ B AP2 LITAF CEBP TATA Luc

2. mtTNF $\beta$ 1-pGL3basic .991  
(-991 to 1 Δ-515 to -511)  
NF- $\kappa$ B AP2 CEBP TATA Luc

3. mtTNF $\beta$ 2-pGL3basic  
(-550 to -487 plus TATA box)  
LITAF TATA Luc

4. mtTNF $\beta$ 3-pGL3basic (-550 to  
-487 Δ-515 to -511 plus TATA box)  
TATA Luc

**FIG. 1**

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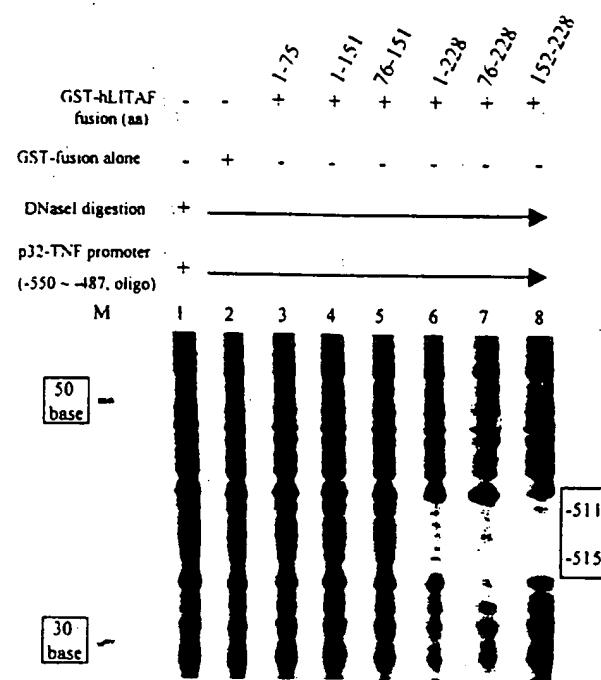


FIG. 2

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-550 \*\*\*\*\* -487  
5' AGGCCTCAAGCCTGCCACCAAGCCCCCAGCTCCTTCTCCCCGAGGGACCCAAACACAGGCCTCA-3'

FIG. 3

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		1 - 151	152 - 228	1- 228		
GST-hLITAF fusion (aa)	-	+	+	+	+	+
GST fusion alone	-	+	-	-	-	-
Unlabeled competitor	-	-	+	-	+	-
<sup>32</sup> P-hTNF $\alpha$ promoter (-550~ -487, oligo)	+	+	+	+	+	+
	1	2	3	4	5	6
					7	8



FIG. 4A

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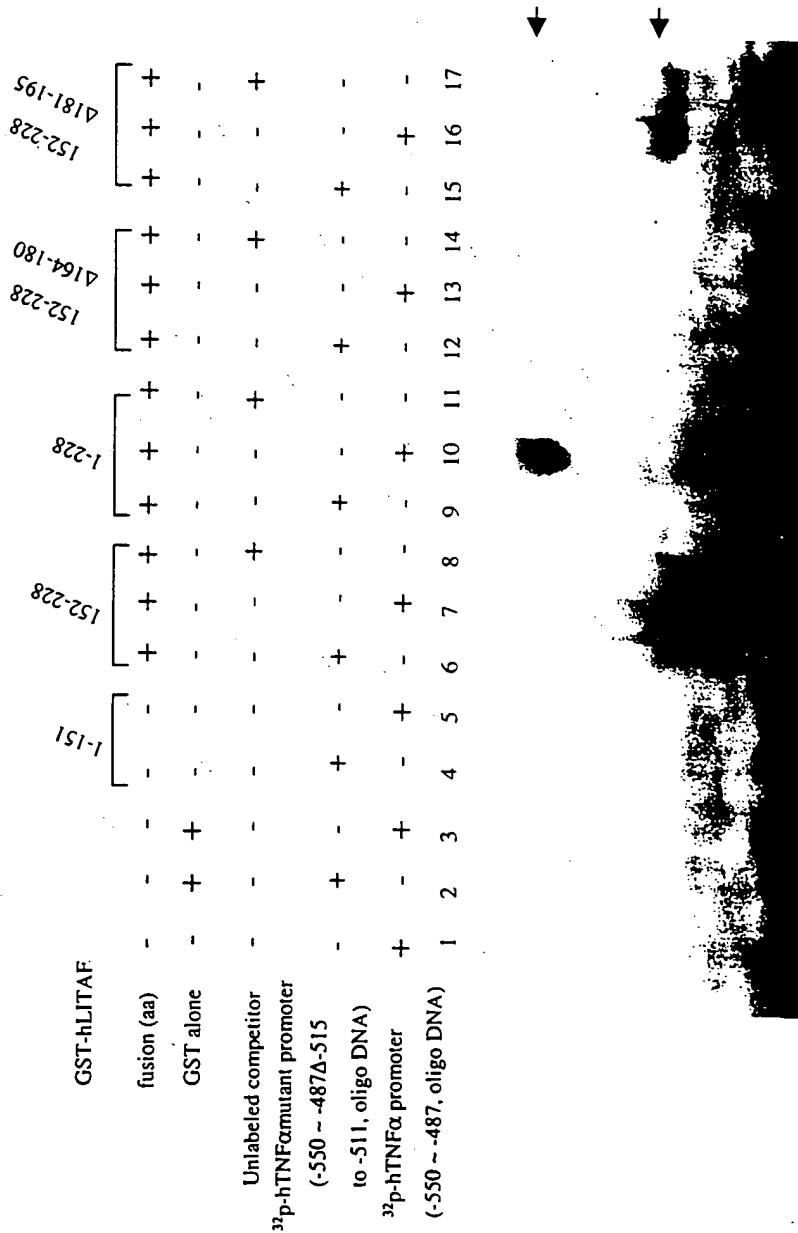


FIG. 4B

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TNF- $\alpha$  secretion upon stimulation by HA peptide or  
hLITAF peptide A, B, C for 24 hrs in THP-1 cells.

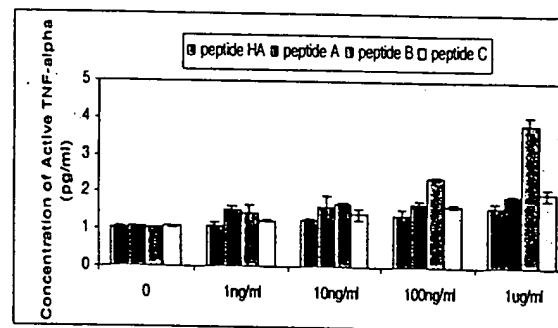


FIG. 5

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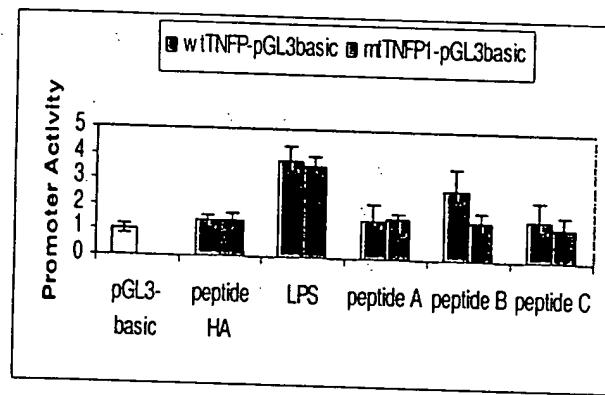


FIG. 6A

**Title: NOVEL LITAF BINDING SITE PEPTIDES AND METHODS OF USING THE SAME**

**APPLICANTS OR GOING**

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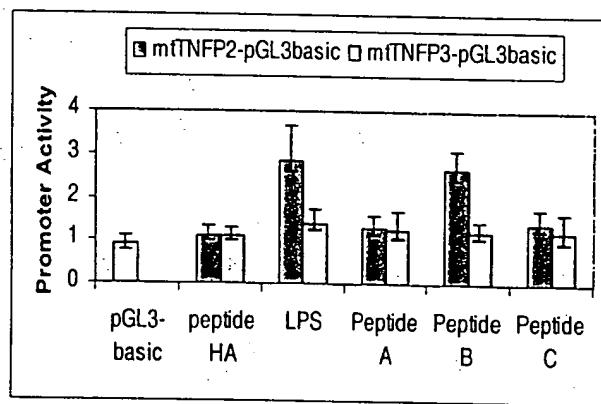
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**FIG. 6B**

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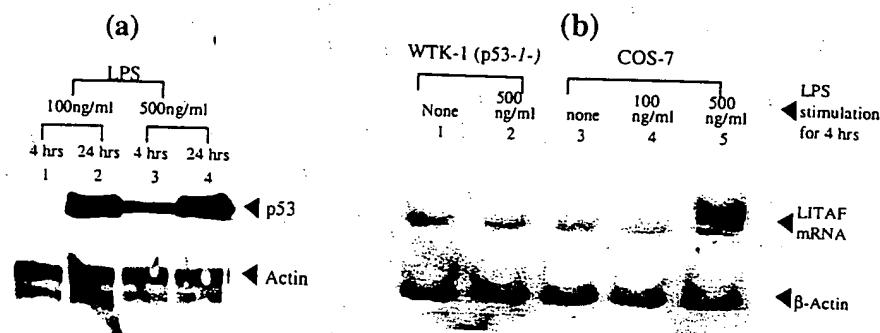
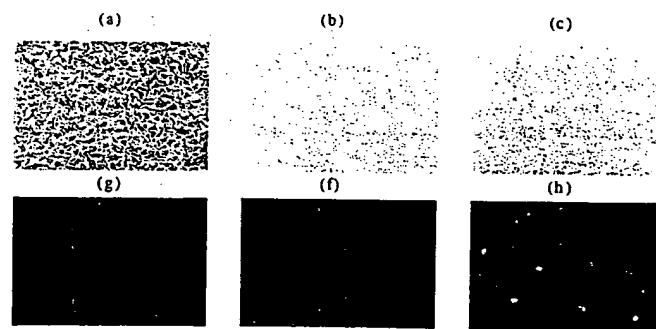


FIG. 7

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**FIG. 8**

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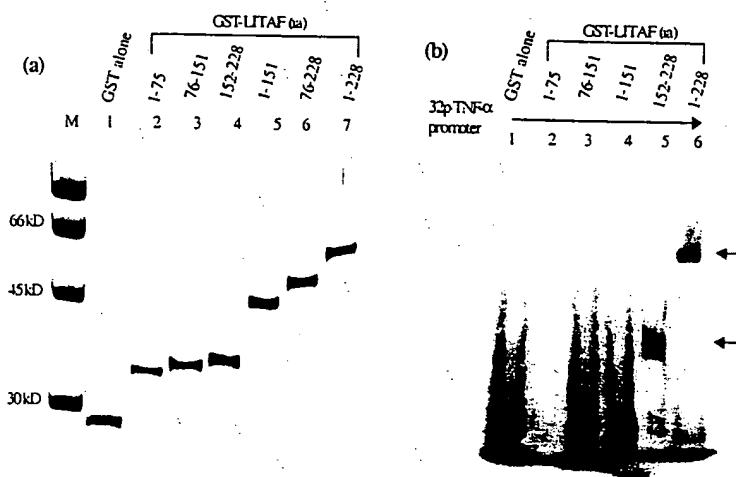


FIG. 9

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TNF- $\alpha$  promoter/pGL3basic constructs.  
 The binding site of transcription factor  
 on promoter is indicated by "□"

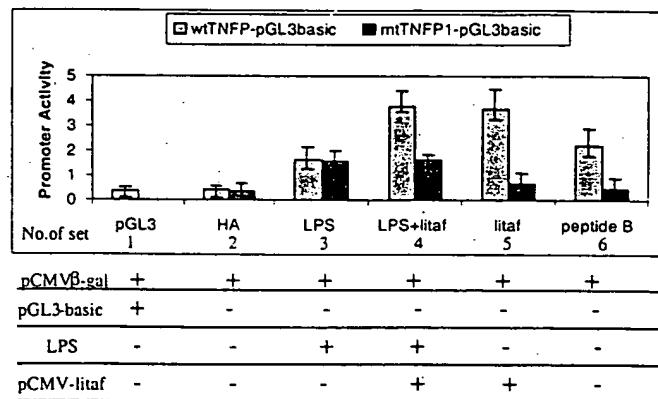
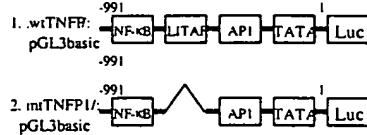


FIG. 10

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<210> SEQ ID NO. 1:  
<211> LENGTH: 228  
<212> TYPE: PRT  
<213> ORGANISM: Homo Sapiens  
<400> SEQUENCE: 1

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Thr Gly Pro Ser Ser Ala Pro Ser Ala Pro	20
Pro Ser Tyr Glu Glu Thr Val Ala Val Asn	30
Ser Tyr Tyr Pro Thr Pro Pro Ala Pro Met	40
Pro Gly Pro Thr Thr Gly Leu Val Thr Gly	50
Pro Asp Gly Lys Gly Met Asn Pro Pro Ser	60
Tyr Tyr Thr Gln Pro Ala Pro Ile Pro Asn	70
Asn Asn Pro Ile Thr Val Gln Thr Val Tyr	80
Val Gln His Pro Ile Thr Phe Leu Asp Arg	90
Pro Ile Gln Met Cys Cys Pro Ser Cys Asn	100
Lys Met Ile Val Ser Gln Leu Ser Tyr Asn	110
Ala Gly Ala Leu Thr Trp Leu Ser Cys Gly	120
Ser Leu Cys Leu Leu Gly Val His Ser Gly	130
Leu Leu Leu His Pro Leu Leu Arg Gly Cys	140
Pro Ala Gly Arg Gly Pro Leu Leu Ser Gln	150
Leu Gln Ser Ser Pro Gly His Leu Gln Ala	160
Phe Val Gly Leu Ser Gln Thr Trp Arg Glu	170
Pro Gly Ala Ala Gly Ser Pro Phe His Leu	180
Ser Ser Ser Phe Thr Pro Gly Gly Ser	190
Ala Leu Val Val Ser Pro Leu Gln Gly Ala	200
His Leu His Val Phe Phe Trp Gly Glu Tyr	210
Val Ala Lys Leu Thr Asn Leu Gln Thr Pro	220
Glu Ile Ala Ala Trp Ser Arg Ala	228

FIG. 11

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<210> SEQ ID NO. 2:

<211> LENGTH: 1773

<212> TYPE: DNA

<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 2

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cctcccccgcg gggtaaggcg ggcaccccgcc gagcgcaggg gtcctcttac tgctgtatggc 180  
acccagctct gggcccagac gccgctcacc gtccaccgc ggtgctgggt aaaatgtcgg 240  
ttccaggacc ttaccaggcg gccactgggc cttcctcagc accatccgca cctccatcct 300  
atgaagagac agtggctgtt aacagttatt accccacacc tccagctccc atgcctggc 360  
caactacggg gcttgtgacg gggcctgatg ggaagggcat gaatcctcct tcgtattata 420  
cccagccagc gcccataccc aataacaatc caattaccgt gcagacggc tacgtgcagc 480  
accccatcac cttttggac cgccctatcc aaatgtgttg tccttcctgc aacaagatga 540  
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ggaggattta taaaaccatt ttctgtatc aaatgattgg tgtcattttc ccatttgcca 1740  
atgttagtctc actaaaaaaaaaaa aaa 1773

FIG. 12